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Plant feeding site selection on soybean by the facultatively phytophagous predator *Orius insidiosus*

Christine A. Armer[1,*], Robert N. Wiedenmann[2] & Daniel R. Bush[3]
[1]*Department of Entomology, University of Illinois, Urbana, IL 61801, USA;* [*]*Current address: Department of Entomology, Oregon State University, Corvallis, OR 97331 USA;* [2]*Center for Economic Entomology, Illinois Natural History Survey, Champaign, IL 61820, USA;* [3]*Photosynthesis Research Unit, USDA-ARS, and Department of Plant Biology, University of Illinois, Urbana, IL 61801, USA*

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Abstract

Experiments were conducted to test whether the facultatively phytophagous predator *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) ingested phloem, xylem or mesophyll contents from soybean plants (*Glycine max* L.). Potential uptake of phloem sap was examined by radiolabeling photosynthate with $^{14}CO_2$ and then measuring the accumulation of radiolabeled metabolites in feeding animals. Most *O. insidiosus* feeding on radiolabeled plants ingested no or very low levels of label; only 3% ingested small amounts of label, indicating the experimental insects fed very little, if at all, on the phloem. In contrast, well known phloem feeding insects used as positive controls accumulated substantial levels of labeled metabolites after feeding on known host plants. *O. insidiosus* did feed on xylem contents, as shown by ingestion of safranin-labeled xylem fluid. A few of the insects showed signs of feeding on the mesophyll, as indicated by the presence of chloroplasts in the gut. However, the small diameter of the food canal may cause limited passage of chloroplasts, which would contribute to an underestimation of the frequency of mesophyll feeding. Some radiolabeled metabolites remain in the mesophyll so those insects that ingested low levels of radiolabel probably ingested label from the mesophyll, which supports the notion that some level of mesophyll feeding occurred. Feeding site determines the nutrients ingested during phytophagy. These insects obtain water from the xylem, and may ingest small amounts of starches, sugars, and amino acids from the mesophyll. The results suggest that facultative phytophagy by this heteropteran predator primarily provides the insect with water, but also may provide some nutrients that supplement a prey diet and help the predator survive periods when prey are scarce.

Introduction

The insidious flower bug, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae), is the most common and effective predator of mites, thrips, and lepidopteran eggs in soybeans, *Glycine max* L. (Robinson et al., 1972; Bechinski & Pedigo, 1981; Brown & Goyer, 1984; Marston et al., 1984). This predator also feeds on plants, even when prey are readily available (Armer, 1996). Facultative phytophagy is important in biological control as it allows for maintenance of predator populations during periods of prey scarcity (Wiedenmann & O'Neil, 1991, 1992; Naranjo & Gibson, 1996; Coll, 1997). Knowledge of the plant feeding site and nutrients obtained during phytophagy may indicate what benefit *O. insidiosus* gains from ingesting plant materials, which may help us manipulate this natural enemy to our advantage.

A wide variety of nutrients is available to phytophagous insects at different sites in plants. Starch and lipids are stored in the mesophyll. Phloem sieve tubes carry high concentrations of sugars and low concentrations of proteins and amino acids (Giaquinta, 1983). Sucrose is present in high concentrations (up to 1 M, or 10–20% weight by volume) in the phloem extract of some legumes, but is absent in the xylem sap (Pate et

al., 1975; Giaquinta, 1983; Geiger et al., 1973). Fisher (1978) found the soybean phloem contains up to 11.5% sucrose by weight, which is in agreement with Pate et al.'s (1975) results from other legumes. Total amino acids range from 16 to 40 mg/ml in the phloem sap, and only 0.5 to 2.4 mg/ml in the xylem fluids of several legumes (Pate et al., 1975).

Identification of plant feeding sites would clearly indicate what nutrients the insect is utilizing to supplement its diet. By extension, we then would know the importance of plant feeding to facultatively phytophagous predators, such as *O. insidiosus* and other heteropterans, especially in times of prey scarcity (Wiedenmann et al., 1996; Naranjo & Gibson, 1996). In this study, we examined *O. insidiosus* feeding on soybean to determine whether it fed in the phloem, xylem and/or mesophyll of the soybean plant.

Materials and methods

Plant and insect culture. Soybean plants were grown in the greenhouse year-round. All plants were grown from 'Pioneer 9183' seed produced in 1992. Feeding site experiments were performed in 1995 and 1996. Plants were moved from the greenhouse to the growth chamber ca. 30 days after planting, when the plants were at V3–V6 stages (Fehr et al., 1971), or approximately four to six nodes tall. Plants were held in a growth chamber up to one week before use in feeding site studies.

Orius insidiosus were reared in a colony maintained in 0.9-liter yogurt containers topped with screening. Freeze-killed eggs of *Trichoplusia ni* (Hübner), *Helicoverpa zea* (Boddie), and *Heliothis virescens* (F.) were provided every 5 days as food. Fresh green bean (*Phaseolus vulgaris* L.) pieces were provided as a moisture source and an oviposition site, and were moved to new containers for nymphal emergence every 5 days. Insect colonies and plants were kept in a growth chamber on a cycle of L16:D8 hours, and 24:18 °C. Relative humidity ranged between 70–80%. Two 40-watt General Electric fluorescent wide-spectrum plant and aquarium bulbs provided light to each plant bench in the growth chamber.

Phloem radiolabeling. Phloem sap can be radiolabeled preferentially with ^{14}C sugars derived from $^{14}CO_2$ uptake. Four trials were run during the phloem labeling experiments. *O. insidiosus* was tested, as were *Myzus persicae* (Sulzer) (Homoptera: Aphididae), and *Circulifer tenellus* (Baker) (Homoptera: Cicadellidae), two known phloem-feeders comparable in mass to *O. insidiosus*. No phloem-feeding insects found in North America colonize soybean plants. Although the aphid and leafhopper cannot reproduce on soybeans, they have broad host ranges and might feed to a limited extent on soybeans. The latter two species were used as positive controls to indicate the approximate amount of label that *O. insidiosus* could ingest if it were to feed on the phloem. The homopterans were from laboratory-reared colonies from the Illinois Natural History Survey, Champaign. The aphids and insidious flower bugs were adults, whereas the *C. tenellus* were both older nymphs and adults. The first two trials involved only *O. insidiosus*. The third trial examined feeding by *O. insidiosus*, *M. persicae*, and *C. tenellus*. Neither of the known phloem-feeding species took up label from the soybeans, and so a fourth trial was added which examined feeding by only the aphids and leafhoppers on ^{14}C-labeled sugar beets (*Beta vulgaris* L.), a known host plant for both species.

The radiolabeling trials were set up with individual greenhouse-grown plants. Soybeans were at stages V3–V4 (Fehr et al., 1971), whereas the sugar beet plant used in the last trial was six nodes tall. The plant to be labeled was set up with a light source 22.5 cm above the plant, providing 55 000 lux illumination (measured with a Lutron LX-101 luxmeter) at the uppermost node. An infrared shield was placed 9.5 cm above the top leaves, between the plant and the light source. Each plant was enclosed in a clear polyethylene bag, which was taped tightly around the plant's stem just below the bottom node to keep the labeled CO_2 from leaking into the soil. An open vial containing 50 μl ^{14}C-labeled sodium bicarbonate (214.6 mBq/mmol specific activity, NEN Products) was taped to the inside of the bag before the bag was securely closed. A small slit was then made in the bag to insert a pipette with 1 ml 1M HCl, which combined with the sodium bicarbonate to release 50 μC $^{14}CO_2$. The plant took up labeled $^{14}CO_2$ for 15 min. The bag was then removed and the plant allowed to take up atmospheric CO_2 for 5 min before the insects were placed on the plant to feed. At a translocation rate of 1.5 cm/min (Vernon & Aronoff, 1952), a 15-min exposure was considered adequate for the label to spread throughout the plant.

O. insidiosus adults, which were deprived of food and water for ca. 24 hours, were placed individually in clip cages on the leaves in trials 1–3. Clip cages were made from short pieces of clear butyerite plastic tubes with gauze glued over one end. The open end

was rimmed with a ring of high density foam rubber. Each cage was held on to a plant leaflet with a bent hair clip, with a thin wooden stake on the other side of the leaflet to support the clip cage. The opening of each cage was 1 cm in diameter. Insects also were caged on the petioles in the first trial. Leafhoppers and aphids used in trials 3 and 4 were starved for about 2.5 hours. Aphids starved long periods may not feed normally (G. E. Kampmeier, pers. comm.). The soybean plant used in trial 3 was rubbed with a plant extract of sugar beet leaf ground in water to simulate the host plant of the aphids and leafhoppers. Insects were allowed to feed for one hour in each trial. The direct light source was kept on during insect feeding in trials 1, 3, and 4, but was turned off so the ambient room lighting provided the only light during feeding in trial 2. As a control, five *O. insidiosus* were allowed to feed on an unlabeled plant, and were examined for radioactivity in the same manner as the treatment insects.

After feeding, insects were frozen immediately, and then ground in Cytoscint ES scintillation fluid, and accumulated radiolabel was measured with scintillation spectroscopy. Samples from leaves on which the insects fed were collected with a 4.5 mm cork borer, and were frozen, ground, and tested for label. Petiole and stem samples were also taken from trial 1 and were treated in the same manner as the leaf samples. All leaf samples were taken immediately after the clip cages were removed. The leaf sample was taken at or near the location of the corresponding clip cage. The mass of *M. persicae* and *C. tenellus* was measured on a CAHN C-31 microbalance to compare them to the average mass of *O. insidiosus*. The leaf disks in trials 3 and 4 also were weighed to compare insect mass and label with that of the leaf disks. Although the clip cages made direct observations of feeding impossible, an estimate of feeding time was calculated by noting the approximate percentage of time each insect remained on the leaf rather than the walls of the clip cage.

Xylem staining. Safranin O is an acidic red stain, which is selectively translocated in the xylem (Khan & Saxena, 1984). We examined dyed leaves under the dissecting microscope to determine dye distribution patterns. Although most dye was found in the vasculature, some dye did spread into the mesophyll immediately surrounding the veins in about 35% of the leaves. Dye uptake time, from one to three hours, did not appear to make a difference in the amount of dye that spread into the mesophyll immediately surrounding the veins. Although it is not possible to exclude the possibility of dye movement into the phloem, most of the dye was concentrated in the xylem, as determined by microscopic examination of cross sectional plant pieces.

Soybean plants used were between 28 and 32 days old and at stages V3–V6 (Fehr et al., 1971). The stem with the top three nodes of the plant was cut and placed in an aqueous 0.1% Safranin O solution. A 5-watt fan placed 8 cm from the plant blew air across the leaves to help keep the stomata open, causing the plant to take up dye. A light source was placed 20 cm above the top of the leaves. A clear glass Pyrex pan ($21 \times 21 \times 5$ cm) filled with a 1% aqueous solution of copper sulfate was placed between the light source and the plant to block infrared radiation and excess heat. As in the phloem-labeling experiment, 55 000 lux of light reached the leaves. The plants took up dye solution for 1–3 hours. At the end of the staining period, the plant stems were placed in tap water. *O. insidiosus* males and females that had been deprived of food and water for ca. 24 hours were then placed individually in clip cages on the dyed leaves. The thirteen insects used in the first replicate were from the laboratory colony, whereas those in the other four replicates were field-collected during the summer of 1996 from alfalfa fields in Urbana, IL. A total of 67 individuals was examined. Additionally, fifteen control insects were placed in clip cages on plant cuttings held in tap water with no dye. The experimental and control insects were allowed to feed for one hour and were examined periodically to determine which individuals were feeding. The insects were then sealed into cones made of filter paper. The cones were held in a growth chamber at 24:18 °C, L16:D8 and 70% r.h. for three days, at which point all of the insects had died. The filter papers were examined for safranin O dye in the excreta of the insects. The insects were also dissected and examined for traces of non-excreted dye. A visual rating scale of 0–3 was created to estimate the amount of xylem feeding: a rating of 0 indicated no dye was found in the excreta or the dissected insect; a rating of 1 indicated a small amount of dye, less than 0.5 mm^3 color, was found either in the excreta or in the insect; a rating of 2 indicated a small amount of dye was found in both the excreta and the insect; and a rating of 3 indicated larger amounts of dye, 1 mm^3 or more, were found in the excreta and the insect. The precise amounts of dye could not be determined because the dye could become concentrated or diluted in the insect's body. The rating scale instead provides information on relative amounts of xylem feeding. All leaflets where insects had fed were examined under the

dissecting microscope to determine if dye had spread into the mesophyll.

Dissections for chloroplasts/mesophyll feeding. Insects can be dissected and the alimentary canal examined for the presence of chloroplasts, which are specific to the mesophyll. Hunter & Backus (1989) used thin-layer chromatography (TLC) to show that the green color seen in potato leafhoppers is due to ingestion of mesophyll and chloroplasts, rather than the presence of a green pigment in the hemolymph. Unlike leafhoppers, *O. insidiosus* does not have green pigments in the hemolymph, and so simple dissections can indicate if individuals have ingested chloroplasts. A total of 101 *O. insidiosus* was used for the mesophyll-feeding tests; 72 were field-caught, and 29 were from a laboratory colony. Ingested food will pass through the gut of *O. insidiosus* in 24 hours (pers. obs.), so any chloroplasts that may have been ingested in the field or in laboratory culture would have been excreted prior to examining the insects for soybean chloroplast ingestion. All insects were held in cages with *T. ni* eggs and green bean pieces prior to placing them on soybean leaves for the experiment. Fifty-seven *O. insidiosus* were individually caged on soybean leaves in the growth chamber and allowed to feed for two to eight days. During the feeding period, 12 insects died. An additional 44 insects were allowed to feed individually in clip cages until they died. All 101 insects (56 dead, 45 alive) were dissected and examined for chloroplasts. Five control insects fed water and *T. ni* eggs, and kept without any plant source for 5 days also were dissected. Live insects were chilled briefly at −80 °C immediately before dissection.

Results

Phloem radiolabeling. Control insects fed on unlabeled plants had less than 10.0 cpm radioactivity, with a mean of 4.0 cpm (S.E. = 1.57). Using the control values, we established the criterion that insects with less than 15 cpm (mean plus 3 S.D.) were considered not to have fed on phloem (P = 0.001, *Z*-test). Of 107 *O. insidiosus* exposed to radiolabeled soybean, the mean value was 13.2 cpm (S.E. = 3.8). A total of 87% of *O. insidiosus* examined ingested less than 15 cpm, including more than 30% that took up zero radiolabel; another 4% ingested between 15 and 40 cpm, and 3% ingested between 40 and 85 cpm (Figure 1). Three individuals, or 3% of those examined,

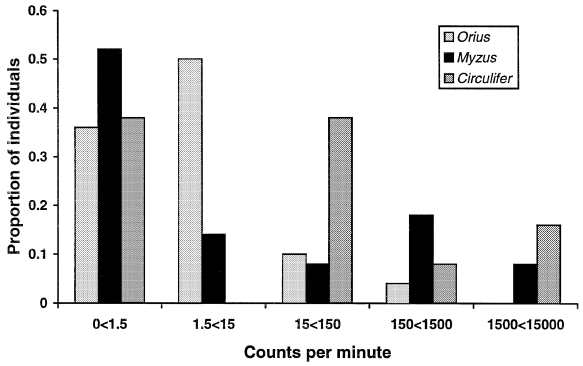

Figure 1. Amount of radiolabel (cpm) ingested by *Orius insidiosus* allowed to feed on radiolabeled soybean, and *Myzus persicae* and *Circulifer tenellus* allowed to feed on radiolabeled soybean and sugar beet plants for one hour.

ingested more than 150 cpm (Figure 1); these three individuals took up 159, 169, and 325 cpm. These three insects were from trial 2, in which the bright lighting source was not used during feeding. No differences were apparent between the label counts for the two light levels during feeding (unpaired *t*-test, StatView 4.01, df = 51, t = 1.462, P = 0.15), so the data are combined in Figure 1. Insects observed feeding during the one hour trial ingested more label than those not seen feeding (regression analysis, StatView 4.01, $r^2 = 0.18$, P = 0.04). However, 17 of the 23 insects observed apparently feeding on the leaf had radiolabel counts of 10 cpm or less, and so were considered not to have ingested label.

Radiolabel levels in leaves at feeding sites (Table 1) averaged 13 505 cpm per leaf disk of average mass 1.5 mg (S.E. = 1016), so an insect feeding at the appropriate site could have ingested large amounts of label. The leaves fed on by the three most highly-labeled *O. insidiosus* contained label within the range of label found in the other leaves. Label levels in the stem dropped from 32 091 cpm per stem section of mass 1.5 mg between two of the labeled nodes to 18 648 cpm immediately below the bottom labeled node, to 9529 cpm approximately 1 cm down the stem below the bottom labeled node, to 5977 cpm 1 cm further down. This rapid decrease in label levels below the labeled nodes indicates the label did not rapidly exit the leaves and petioles to travel to sink regions such as the roots.

The phloem feeders *M. persicae* and *C. tenellus* both took up large amounts of label when they fed on sugar beet, their preferred host plant. Several individuals of each species accumulated between 2000 and

Table 1. Amounts of radiolabel (mean counts per minute ± S.E.) found in soybean and sugar beet, and derived from phloem ingested by three insect species

Trial	Plant source	Plant radiolabel (cpm/leaf disk)	Insect species	Insect radiolabel (cpm/insect)
control	unlabeled soybean	–	*O. insidiosus* ($n = 5$)	4.0 ± 1.6 (range 0-10)
1	soybean ($n = 47$)	15,027 ± 1682 (range 2731-66,622)	*O. insidiosus* ($n = 50$)	7.7 ± 1.6 (range 0–62)
2	soybean ($n = 27$)	11,172 ± 605 (range 4238–18,670)	*O. insidiosus* ($n = 52$)	19.0 ± 7.7 (range 0–325)
3	soybean ($n = 22$)	6355 ± 814 (range 1812–16,423)	*O. insidiosus* ($n = 5$)	8.8 ± 5.3 (range 0–29)
			M. persicae ($n = 11$)	5.0 ± 2.4 (range 0–23)
			C. tenellus ($n = 5$)	22.0 ± 7.1 (range 0–44)
4	sugar beet ($n = 9$)	5864 ± 1780 (range 495–13,773)	*M. persicae* ($n = 16$)	524.7 ± 244.2 (range 0–3429)
			C. tenellus ($n = 8$)	1396 ± 973.9 (range 0–7856)

Leaf disks were 15.9 mm^2. Soybean leaf disks weighed approximately 1.5 mg each, and sugar beet leaf disks weighed approximately 3.9 mg each.

8000 cpm of ^{14}C-labeled metabolites (Table 1). Mean levels of radiolabel ingested were 525 cpm/individual (S.E. = 244 cpm) by *M. persicae*, and 1396 cpm (S.E. = 974 cpm) by *C. tenellus* individuals. When placed on soybeans, a non-host plant, these species accumulated less than 50 cpm (Table 1). The amount of label found in leaf disks from soybean (disk mean weight 1.5 mg) and sugar beet leaves (disk mean weight 3.9 mg) was not significantly different (unpaired t-test, StatView 4.01, df = 134, $t = 1.571$, P = 0.1186).

The mass of the average *O. insidiosus* differed significantly from that of both *M. persicae* and *C. tenellus* (StatView 4.01 unpaired t-test; $P < 0.01$ for all), but the masses were within an order of magnitude, with *C. tenellus* having a mass of 0.33–1.16 mg, *O. insidiosus* 0.32–0.42 mg, and *M. persicae* 0.10–0.40 mg. When normalized for body mass, *O. insidiosus* feeding on soybean ingested 23.7 cpm per mg mass, whereas on sugar beet, *C. tenellus* ingested 1813.0 cpm per mg mass, and *M. persicae* ingested 2386.4 cpm per mg mass.

Xylem staining. Of the 67 *O. insidiosus* examined for xylem-feeding, 28 (42%) contained some dye and/or excreted dye (Figure 2). The amount of dye excreted or found in the abdomen of dissected insects varied greatly. One insect, with a rating of 3, fed extensively and contained large amounts of dye in the abdomen and the excreta. Ten insects fed moderately on the xylem, as indicated by dye in both the insect and the excreta, with a rating of 2. Seventeen insects fed less on the xylem or may have ingested small amounts of xylem fluid while probing, as indicated by a rating of 1. About half of the insects that took up dye were feeding on leaves in which no dye had spread into the mesophyll cells, so they were clearly feeding on the vascular tissues. No differences were found in dye uptake between the lab colony insects and field-caught insects (unpaired t-test, StatView 4.01, df = 12, $t = 0.414$, P = 0.69). The results from field-caught and lab-reared insects were combined in Figure 2. Thirteen of the 44 insects which appeared to be feeding while caged for one hour on the dyed plants ingested dye. Additionally, several insects which fed briefly between observation periods did ingest dye. The one insect which took up high levels of dye was not observed feeding. Thus, some insects could take up xylem fluid and dye rapidly during short feeding bouts. Other insects which appeared

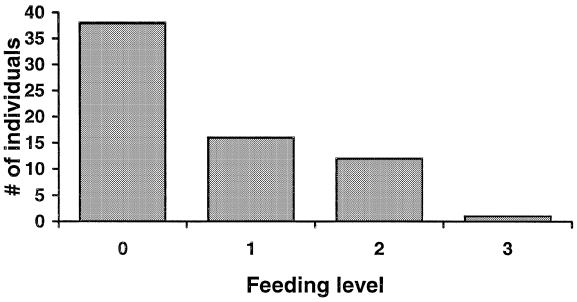

Figure 2. Levels of soybean xylem feeding by *Orius insidiosus* as indicated by the presence of safranin O dye in the insect or its excreta. The feeding level scale indicates: 0 – no feeding; 1 – small amounts of dye in either excreta or dissected insect; 2 – small amounts of dye in both excreta and insect; and 3 – higher levels of dye in both excreta and insect.

to be feeding throughout the one hour period did not feed on the xylem. The control insects fed on undyed plants contained no hints of red.

Dissections for chloroplasts/mesophyll feeding. Of 101 individuals examined (33 males and 78 females), two females that had fed for 8 days exhibited a green color in the posterior of the abdomen, indicating they had ingested chloroplasts. None of the insects contained green color in the fore- or mid-gut. Sixty-six of the insects had died before dissection, and the varying degree of desiccation made finding the alimentary canal difficult during dissection. However, the green chloroplasts should have been visible in these insects if they had ingested any chlorophyll. None of the control insects, which did not feed on plants, had any green color in the abdomen.

Discussion

Plant feeding by *O. insidiosus* and other heteropteran predators has been examined previously for effects on survival and longevity. Ridgway & Jones (1968) concluded that the two heteropteran predators *Geocoris pallens* (Stål) and *Nabis americoferus* (Carayon) fed on plants for moisture. However, Stoner (1972) found that three *Nabis* species had increased survival on plant food over water alone. None of the *Nabis* studied developed beyond the first instar without prey, and Stoner concluded that *Nabis* was probably more predaceous than was *Geocoris*. *Orius tristicolor* (White) nymphs were found to mature on green beans alone (Salas-Aguilar & Ehler, 1977). However, although Kiman & Yeargan (1985) reported that *O. insidiosus* matured from neonate to adult on a diet of pollen, they found that this insect could not develop past the second instar when fed only green beans. Ridgway et al. (1967) noted that *O. insidiosus* and other heteropteran predators were poisoned by systemic pesticides. Other than this last report, no studies have considered on which plant tissues *O. insidiosus* feeds, and what nutrients this insect may obtain from plant feeding.

Previous investigations of phytophagous insect feeding sites used leaf tissue sectioning to view either the mouthparts or feeding tracks in the plant tissues. Prior to the above studies, we examined feeding tracks by staining and with confocal microscopy, which provided no clear evidence of feeding site. Results based on tissue sectioning are prone to misinterpretation because of the potentially large numbers of artifacts. For example, tissues torn during sectioning may closely resemble, and be mistaken for, true feeding tracks. Mouthparts inserted in tissues may move during sectioning, so their location may not indicate where the insect actively was probing. Additionally, the mouthparts and tracks indicate where the insect probed, but not necessarily where it fed (Hunter & Backus, 1989). To overcome these limitations, we attempted to examine directly the materials ingested to determine on what tissues *O. insidiosus* fed.

The phloem contents can be preferentially labeled by $^{14}CO_2$ uptake. Carbon dioxide is taken into the plant through the stomata and enters the mesophyll cells where it is reductively assimilated in the chloroplast and sucrose is subsequently synthesized in the cytoplasm (Taiz & Zeiger, 1991). As much as 80% of the assimilated carbon dioxide is then exported from the leaf via the phloem to satisfy the nutritional needs of the plants' heterotrophic tissues (Hume & Criswell, 1973; Bush 1993). Thus, an insect that feeds on the phloem of a $^{14}CO_2$ labeled plant should accumulate substantial levels of radiolabeled assimilate. Previous studies involving radiolabeling soybean plants with $^{14}CO_2$ showed that the label was concentrated in the phloem (Perkins et al., 1959; Nelson, 1962; Thrower, 1962; see also Biddulph et al., 1958 on kidney beans). Low levels of radiolabel may be found in the mesophyll, which acts as either source or sink tissue. We used the amount of labeled metabolite accumulated by a known phloem feeding insect feeding on its host to establish a baseline of expected levels of label accumulation. Comparable levels of $^{14}CO_2$ were assimilated in the leaves of both plants species and since they move most of that material into the phloem, accumu-

lation levels measured in the known phloem feeders provided a good first approximation of expected levels of assimilate accumulation.

Fourteen of the 107 total *O. insidiosus* (13%) allowed to feed on radiolabeled plants contained more than the basal level of 15 cpm, and so acquired small amounts of label. Although the phloem contents are the primary labeling site, the label found in these *O. insidiosus* individuals was low enough to suggest the label may have been picked up on the mouthparts while probing but not ingesting materials from labeled cells, may have been ingested from the apoplastic fluid surrounding all plant cells, or may have been picked up from feeding on the mesophyll. Assimilated sugars are released into the apoplast before being actively loaded into the phloem of the plant's vascular system (Giaquinta, 1983). The three insects that ingested more than 100 cpm label were in a trial in which the bright lights were switched off during insect feeding. Without bright lights, the plants reduce photosynthetic rate, which would reduce the rate at which radiolabeled sugars leave the mesophyll cells where they are synthesized. The three insects could thus have ingested label from the mesophyll before the label reached the phloem. The low levels of labeled assimilate found in *O. insidiosus* when compared to those measured in known phloem-feeders support the idea that these insects acquired the label from one of the regions associated with the mesophyll, where less label is available. Had they fed on the phloem, much higher accumulations would be expected since recently fixed CO_2 is concentrated in the phloem at 0.6 to 1 M sugar (Giaquinta 1983; Geiger et al., 1973). This conclusion was supported by the amount of labeled assimilate accumulated in the known phloem feeders, *M. persicae* and *C. tenellus* (Table 1).

When average body masses are standardized, the phloem-feeding *M. persicae* and *C. tenellus*, used here as positive controls for phloem feeding, ingested up to 100 times as much label as *O. insidiosus*. When fed on sugar beets, a known host plant, *M. persicae* and *C. tenellus* readily ingested between 2000 and 8000 cpm per insect. Twelve percent of *M. persicae* and 25% of *C. tenellus* ingested over 2000 cpm per insect, or nearly an order of magnitude greater than the maximum ingested by *O. insidiosus*. The aphids and leafhoppers weighed between half and three times the weight of an average *O. insidiosus* individual, and so provided a good estimate of the amount of label an insect in that weight range could take up if feeding solely on the phloem. The *O. insidiosus* used in these experiments had been starved without water for 24 hours, and so should feed wherever the highest concentration of nutrients exist. The phloem provides the sugars and amino acids that support growth in the sink tissues of the plant and its contents are more immediately nutritious than the dilute xylem sap or the complex carbohydrates and proteins of the mesophyll. If this insect could feed on the phloem, it would be most likely to during a period of starvation. The insect, if feeding on the phloem, should feed extensively enough to ingest levels similar to those taken up by strict phloem feeders.

An insect can ingest approximately 10% of its body weight if previously starved (A. C. Cohen, pers. com.). The aphids and leafhoppers may have ingested about 0.01–0.1 mg label, which contained up to 7856 cpm. The sugar beet leaf disks contained up to 13 770 cpm (ca. 2993 cpm per mg plant tissue), or less than twice that of a small insect tapping the phloem for just one hour. Approximately 5% of a leaf's volume is occupied by phloem tissues. If all of the label found in the plant was in the phloem, the phloem-feeding insects would have contained far more label. Because these known phloem feeders had reduced levels of radiolabel, we surmise that some of the radiolabel remained in the mesophyll and apoplast during the insects' feeding period. Because the few *O. insidiosus* individuals that ingested label took up low levels, they did not feed on the phloem, but instead fed on the mesophyll or apoplast. Although we cannot be certain that *O. insidiosus* will not feed on phloem tissues in other plants, they clearly did not feed on the phloem in soybean plants.

Forty-five percent of *O. insidiosus* examined ingested some amount of dye from the xylem when feeding for one hour. The safranin O dye may not have remained specifically in the xylem, but if it spread into the phloem, *O. insidiosus* probably would not have ingested it, because the above results indicate this insect does not feed on the phloem to an appreciable extent. If the dye spread into the mesophyll, as occasionally happened, some dye may have been ingested while the insect was feeding from the apoplast. Thus, xylem feeding accounts for most, but not all, of the dye ingestion. The xylem is a dilute stream that consists of amino acids, vitamins, and minerals, but is more than 98% water (Brodbeck et al., 1990). Thus, *O. insidiosus* probably feeds on the xylem primarily to ingest free water, but may also acquire small amounts of amino acids, vitamins and minerals.

Feeding on the phloem or xylem involves ingesting large quantities of water and relatively dilute nutrients. Excretion of water while retaining nutrients requires a

specialized alimentary canal that may include a filter chamber and the loss of Malpighian tubules (Goodchild, 1966). Without these adaptations, extensive feeding on either the xylem or the phloem could be fatal. The increase in fluids would dilute metabolites, which could not be absorbed rapidly enough as wastes passed through the gut. The metabolites would be rapidly excreted, causing death. An insect accidentally tapping the phloem may have sap forced into it by the positive turgor pressure found in the phloem (Goodchild, 1966). Phloem sieve-tubes in soybeans are 10–15 μm diameter with contents under approximately 6 bars positive pressure (Fisher, 1978). From photomicrographs of the stylet imprints in the feeding sheath made by *O. insidiosus*, we estimated the food canal to be approximately 2–3 μm in diameter (Armer, 1996). The smaller diameter of the food canal would cause the pressure to increase to up to nearly 25 times that found in the phloem sieve tube, as pressure equals force divided by the area of the tube. Aphids with food canals as small as 0.6 μm have the phloem contents rapidly forced into the alimentary canal (Mittler, 1957). Thus, phloem feeding, especially for small insects, requires specialized alimentary canal adaptations. The xylem is also a ready source of moisture, but differs importantly from the phloem in having the contents under negative pressure. Therefore, an insect feeding on xylem fluids can actively control the amounts ingested. The alimentary system and Malpighian tubules of *O. insidiosus* have not been examined to determine if the insect is adapted to excrete massive amounts of excess water. However, because it excretes small amounts of rapidly drying frass (pers. obs), this insect probably is adapted to retain as much water as possible. If so, it cannot rapidly resorb metabolites from large quantities of water, and so phloem-feeding could be deadly. Xylem feeding, on the other hand, would not be hazardous to the insect because the insect readily controls the amount of xylem fluid ingested. A pH gradient exists in plant tissues that appears to inform a probing insect of the proximity of the phloem, which has a slightly alkaline pH of 7.2–8.5 (Fife & Frampton, 1936). *O. insidiosus* may use this gradient to actively avoid tapping the phloem. Goodchild (1966) discusses one cimicomorph (an evolutionary grouping to which the Anthocoridae belong) family that has the alimentary system modified to allow feeding on succulent tissues. However, cimicomorphs have not evolved further toward obtaining many nutrients from plant sap. This information suggests that, although *O. insidiosus* feeds on the xylem for moisture and perhaps dilute nutrients, this insect does not ingest much sap volume from the xylem. Although knowledge of this group of insects indicates *O. insidiosus* should not feed on the phloem, three individuals did ingest relatively low but substantial amounts of radiolabel, and could have ingested the label during phloem feeding. The alimentary canal must be examined to determine if this insect has the physical adaptations to survive feeding on the phloem.

Mesophyll feeding is suggested by the dissections as well as radiolabeling. Where *O. insidiosus* fed on the leaf tissues, there was no evidence of stippling, or light patches caused by removal of chloroplasts (Sogawa, 1973). However, *O. insidiosus* may simply not feed in one location long enough to cause stippling (Kabrick & Backus, 1990). Alternately, *O. insidiosus* may not ingest chloroplasts when it does feed on the mesophyll. The food canal of *O. insidiosus* is 2–3 μm diameter. Based on estimations from photographs in Taiz & Zeiger (1991), a common chloroplast ranges in size from 5 to 7 μm. Thus, most chloroplasts are larger than the food canal of *O. insidiosus*. The small diameter of the food canal explains why few individuals consumed whole chloroplasts, though a few small or broken chloroplasts may have been ingested by a large *O. insidiosus* feeding on the mesophyll. The stylets are probably not strong enough or precise enough to actively break chloroplasts into pieces, so few chloroplast pieces would be consumed. Although only 2 of the 101 individuals examined showed color in the gut, probably others fed on the mesophyll but were unable to take up chloroplasts, and simply ingested cell cytoplasm. Radiolabeled CO_2 may be found in the mesophyll cells, which act as source and sink for the label. The minimal levels of radiolabel in most of the insects indicates that few *O. insidiosus* do feed on the mesophyll. However, thirteen percent of insects examined did ingest small amounts of radiolabel. The ingested label likely came from the mesophyll cells where the radiolabeled CO_2 was fixed, indicating that these insects did feed on the mesophyll. Although mesophyll feeding appears not as prevalent as xylem feeding, mesophyll tissues may be an important source of nutrients for *O. insidiosus*, especially when prey are not available.

Cohen (1996) hypothesized that cimicomorph predators rely more on chemical action of their saliva to liquify prey, whereas pentatomomorph predators rely more on mechanical action to feed on prey. Both groups contain predators that are facultatively phytophagous and that appear to feed on plant vasculature (Ridgway et al., 1967). If cimicomorphs, including *O. insidiosus*, primarily utilize enzymes for extraoral digestion, then

examining salivary enzymes would help determine where in a plant the predators are capable of feeding. Knowledge of both salivary enzymes and digestive tract morphology would help indicate how the insect evolved to feed primarily on the xylem and mesophyll, and would determine if plant-feeding provides moisture or nutrients or both. The presence of a salivary amylase is a clear indicator of the capacity to feed on the starch of mesophyll cells. Some heteropterans (Miles, 1959) and, indeed, some cimicomorph predators (Cohen, 1990), produce a salivary amylase. Dissection of salivary glands from an insect as small as *O. insidiosus* would be difficult at best, but would help clarify what nutrients this insect may be obtaining from the mesophyll.

The results presented here show that *O. insidiosus*, a facultatively phytophagous heteropteran predator, feeds primarily on xylem sap and on mesophyll tissues, and rarely, if ever, on the phloem. The nutrients found in field-grown soybeans may differ from those in greenhouse-grown plants, which may affect the time *O. insidiosus* spends feeding on each tissue, but the insect should feed on the same tissues regardless of nutrients in the individual plant. This insect feeds on the xylem and mesophyll, as may other related heteropterans. Because a continuum exists among heteropteran species, ranging from facultatively phytophagous predators to facultatively predaceous phytophages (Wiedenmann et al., 1996; Wiedenmann & Wilson, 1996), it will be critical to understand the benefits of plant feeding to each of the heteropteran taxa. For *O. insidiosus*, facultative phytophagy appears to be the means for obtaining needed moisture to supplement the prey diet, and may provide dilute nutrients during periods of prey scarcity.

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